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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/500,118	09/14/2004	Henrik Semb	034005-008	7902

21839 7590 12/19/2008

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POST OFFICE BOX 1404
ALEXANDRIA, VA 22313-1404

EXAMINER	
TON, THAIAN N	

ART UNIT	PAPER NUMBER
1632	

NOTIFICATION DATE	DELIVERY MODE
12/19/2008	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

ADIPFDD@bipc.com

Office Action Summary	Application No.	Applicant(s)	
	10/500,118	SEMB ET AL.	
	Examiner	Art Unit	
	Thaian N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 November 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-5,9-20,22-56 and 60-65* is/are pending in the application.

4a) Of the above claim(s) *22-34 and 36-56* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-5, 9-20, 35, 60-65* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/17/08 has been entered.

Applicants' Arguments and Response, filed 11/17/08, have been entered. Claims 1-5, 9-20, 22-56, 60-65 are pending; claims 14, 9-11, 14, 16-20, 35, 60-62, 64 and 65 are amended; claims 6-8, 57 and 63 are cancelled; claims 22-34, 36-56 are withdrawn; claims 1-4, 6-11, 13, 14, 16, 17-20, 57-61 are amended; claims 1-5, 9-20, 35, 60-65 are under current examination.

The **Semb** Declaration has been considered.

Election/Restrictions

Applicant's election with traverse of Group I (claims 1-21, 35, 57-61) in the reply filed on 1/12/07 is acknowledged.

Claims 22-34, 36-56 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 1/12/07.

Claim Rejections - 35 USC § 112

The prior rejection of claims 1-20, 35, 57-65 under 35 U.S.C. 112, first paragraph, for enablement is withdrawn in view Applicants' amendment to the claims, which now recite that the human blastocyst-derived cells are cultured on fibroblast feeder cells.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5, 6, 12, 13, 16-20, 62, 64, 65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) as evidenced by Stem Information (National Institutes of Health), when taken with

Rijnders *et al.* and in further view of Lanzendorf *et al.* when taken with US Pat. No. 6,875,607 (published April 5, 2005; filed November 9, 1999). This is a new ground of rejection.

Applicants' have now amended the claims to recite propagating the human blastocyst-derived stem cell colonies by repeatedly passaging the human blastocyst-derived stem cell colonies every 4-5 days, wherein each passaging step comprises manually dissecting the inner homogenous structure of the human blastocyst-derived stem cell colonies to form pieces of the same and placing the pieces on inactivated fibroblast feeder cells, thereby obtaining a stable pluripotent human blastocyst derived stem cell line. The Examiner responds to Applicants' remarks as they pertain to the current rejection.

Applicants' Arguments. Applicants argue that the claims now recite that the human blastocyst-derived cells are "stable" and that one of skill in the art would have recognized that a stable pluripotent human ES cell is one that remains undifferentiated and exhibits no chromosomal instabilities for a period of more than 21 months. Applicants point to p. 7, line 24 to page 9 line 2 of the specification for support for this term. Applicants argue that one of skill in the art would have understood that not only is the generation of large numbers of stable, undifferentiated cells attractive from a commercial point of view, but the ability to produce such large numbers of cells in lots is especially advantageous, because an individual unit may be withdrawn from each lot for characterization of the remaining units within the lot. See p. 14 of the Response. Applicants argue that the state of the art at the time of filing was in chaos because while the establishment of murine ES cells had been well documented for many years, it had not been possible to establish corresponding lines of hES cells. In particular, it was not even known if it would be possible to establish a stable line of hES cells. Applicants argue that in the past, cell lines were not followed for a sufficiently long period of time to establish their true stability, especially chromosomal stability. In particular,

Applicants argue that previously established cell lines did not discuss data pertaining to expression of markers associated with differentiation, and prior to the instant invention, no one had demonstrated a stable hES cell line that remained undifferentiated and exhibited chromosomal stability for an extended period of time. See p. 16 of the Response. Applicants argue that the combined art fail to report the establishment of a stable human ES cell line that remains undifferentiated and chromosomally stable for more than 21 months. Applicants argue that the NIH stem cell information is with regard to proliferation capacity of the H9 cell line, but proliferation capacity is only one of several general indications of having a pluripotent and stable stem cell line, and that this reference does not indicate that the cell line is stable. See p. 20 of the Response.

Response to Arguments. These arguments are considered but not persuasive. The specification, at p. 7, lines 24+ recite that the term "stable" is intended to denote proliferation capacity in an undifferentiated state for more than 21 months when grown on mitotically inactivated embryonic feeder cells. It is noted that neither this definition, nor the present claims recite the limitation that "no chromosomal instabilities" are present in the cells. The Semb Declaration (p. 2, #7) states that the claimed method is capable of generating large numbers of pluripotent human ES cells with sufficient chromosomal stability for passaging for at least 21 months in an undifferentiated state. The Examiner responds by pointing to the NIH document (cited previously) which clearly shows that the H9 has divided for nearly 2 years, and thus, this shows that the cell line has sufficient chromosomal stability for passaging for at least 21 months. Additionally, the NIH document shows that the H9 cell line is capable of proliferating for more than 21 months on mitotically inactivated embryonic feeder cells, and therefore, fulfilling the specification's definition of a "stable" cell line. Finally, the NIH document clearly teaches that ES cells have high telomerase activity, and therefore, do not senesce, whereas differentiated somatic cells stop dividing in culture due to

senescence (p. 2). Accordingly, the NIH document clearly teaches that the H9 cell line is capable of propagation in an undifferentiated state for more than 21 months, which fulfills the limitations of the term "stable" as defined by the instant specification.

Additionally, Applicants point to pages 7-8 for characteristics of ES cells. In particular, the specification teaches that the cells must exhibit proliferation capacity in an undifferentiated state for more than 21 months when grown on mitotically inactivated embryonic feeder cells, exhibits a normal, euploid chromosomal karyotype, maintains the potential to develop into derivatives of all three germ layers both *in vitro* and *in vivo* and exhibits appropriate markers. The Examiner notes that there is no requirement in this definition that the cells exhibit a normal, euploid chromosomal karyotype for 21 months.

The Semb Declaration teaches that while the length of time for which a cell line may be passaged with a high degree of chromosomal stability is not an end in itself, the stability of the cells produced in accordance with the invention allows the production of lots of cells. See p. 2, #7. Additionally, with respect to #8 of the Declaration, the Examiner responds that the claims only require obtaining a stable stem cell line. Thus, the combination of the cited art provides sufficient guidance to show that, for example, the H9 cell line taught by Thomson is considered stable. Accordingly, the cited art of record provides sufficient teachings, guidance and motivation to arrive at the claimed invention.

Applicants' Arguments. Applicants argue that the present claims recite that the blastocyst-derived stem cells are passaged every 4-5 days and that the present inventions have surprisingly found that by combining the steps of manual dissection followed by repeated passaging every 4-5 days, a stable, pluripotent human stem cell line can be obtained. Applicants argue that even though the individual method steps may appear to comprise relatively small modifications, the combined effect of such apparently small changes are unexpected substantial,

allowing for the establishment of highly stable human ES cell lines, which in turn, allows for the production of significant numbers of cells. See p. 15, last ¶ of the Response.

Response to Arguments. These arguments have been fully considered, but are not persuasive. In particular, the claims do not require any particular yield or a significant number of cells. The claims require obtaining a stable, pluripotent human blastocyst-derived stem cell line. The combination of art arrives at this invention with a reasonable expectation of success.

Applicants' Arguments. Applicants argue that Thomson (2001) discuss producing primate ES cells, but only exemplifies producing an ES cell line from the Rhesus Macaque Monkey. Applicants argue that the protocols of Thomson (2001) and Thomson (1998) differ from the instant methods in a number of important respects, firstly, that one of skill in the art would not have reasonably predicted that a method established for isolating and maintaining a culture of pluripotent blastocyst derived stem cells from Rhesus Macaque monkey would succeed when applied to human cells. Additionally, Applicants point to the lack of teaching of mechanical dissection (with a knife or glass capillary) as opposed to being mechanically dissociated with a miropipette. Applicants argue that mechanical dissociation by micropipette implies dissociation of cells the cells into smaller clumps. See p. 17 of the Response. Applicants argue that the instant invention requires passaging the blastocyst-derived cells every 4-5 days, whereas Thomson (2001) and Thomson (1998) do not recite this time frame. See p. 18 of the Response.

Response to Arguments. These arguments have been considered but are not persuasive. In fact, the Thomson (1998) and the newly added reference '607 provide evidence to show that the methods used by Thomson (2001) would reasonably and successfully result in the isolation of ES cells from human blastocysts. See col. 8-9 of the '607 patent with respect to ES cell isolation techniques. Additionally, with respect to Applicants' amendment to the claims which now require manual

dissection, the Examiner notes that the '607 patent clearly teaches cutting of cell colonies to produce pieces which can then be picked up, which clearly fulfills the limitations of the claims, and would refer to a knife or sharp pipette. See col. 18, lines 1-27. The Examiner notes additionally, that the '607 patent fulfills the limitations of the claims with respect to passaging the colonies every 4-5 days by recitation that cells are passaged every 5-7 days (col. 18, line 7).

Rejection

Thomson (2001) teach methods for the production of primate embryonic stem cells, including human ES cells. In particular, they teach the isolation of a blastocyst, isolating cells from the inner cell mass (ICM) of the blastocyst, and plating the ICM cells on embryonic feeder layers (see col. 4, lines 38-49). They teach that these methods can be used in deriving human ES cell lines (col. 7, lines 4-10). In particular, they teach isolating blastocysts from a primate, removing the zona pellucida using pronase, and then removal of the intact inner cell mass cells by gentle pipetting and plated on inactivated, irradiated embryonic fibroblasts (col. 8, lines 32-40). They teach that the dissociated cells are then replated on embryonic feeder layers, and cells demonstrating ES-like morphology are then individually selected and propagated. See col. 8, lines 50-59. They teach the co-culture of human blastocysts with human oviductal cells to produce expanded human blastocysts (see col. 9, lines 23-32). Thomson teach that their human ES cell line is stable (see issued claim 1). They further teach using mouse feeder cells, and particular, mouse STO cells (ATCC 56-X), which are irradiated mouse fibroblast feeder cells.

Thomson (1998) provide specific guidance to show that the production of human ES cells requires human embryos, which are produced by IVF (see p. 1147, col. 2, #6). With regard to claim 20, although Thomson (1998) teach that their cells have been passaged for more than 8 months, they do not specifically teach that the

cells have a proliferation capacity, in an undifferentiated state, for more than 21 months, as required by part i) of claim 20. However, The NIH stem cell information provides evidence that the exact cell line described in the Thomson (1998) paper, the H9 cell line, has the proliferation capacity for more than 21 months, stating that, "[T]he H9 cell line has divided for nearly two years *in vitro*." See page 2, first sentence. Thus, this citation describes an inherent property of the cells, and shows that they have a proliferation capacity of over 21 months. Furthermore, Thomson (1998) provide the various characteristics required by the claim 20, namely that the ES cell lines exhibited a normal karyotype (see page 1145, 2nd col.) (see part (ii) of the claim 20); had the developmental potential to form the derivatives of all three germ layers, both *in vitro* and *in vivo* (see Abstract, and page 1146, 1st col., 2nd full ¶) (part iii of the claim); exhibited appropriate markers, including expression of SSEA-3, SSEA-5, TRA-1-60, TRA-1-81 (p. 1145, 3rd col., last ¶), as required by part (iv) of the claim; did not express SSEA-1 (p. 1146, 1st col., 1st ¶); formed teratomas when injected into immunocompromised mice and is capable of differentiation (p. 1146, 2nd full ¶), as required by steps vi-vii of the claim.

Neither Thomson (2001), nor Thomson (1998) teach using a fertilized oocyte having a grade 1 or 2 to obtain a blastocyst of grade A or B, as recited in step (i) of claims 1-3. The instant specification defines Grade 1 fertilized oocytes as those which have even blastomers, with no fragments, and Grade 2 fertilized oocytes as those with <20% fragments (see page 4, lines 1-52); Grade A blastocysts are those with expanded distinct inner cell mass cells 5-7 days after fertilization, and Grade B blastocysts are not expanded but, otherwise like Grade A (p. 4, lines 10-11). Rijnders *et al.* provide specific guidance with regard to the identification of class I and class II embryos, which are defined similarly as the instant specification's definition (see p. 2870, 1st col., Embryo Pre-selection and Selection) and teach that class I and II embryos produced a higher percentage of blastocysts, and had less embryos that arrested in development of degenerated (see Abstract). Lazendorf *et*

al. teach methods of identification of Grade 1 and 2 (analogous to the specification's definition of Grade A and B) blastocysts and they teach the hatching of the blastocysts (see page 134, 1st col.). They teach that some of the embryos, which were grade 1, hatched spontaneously, and that one blastocyst was grade 2, and required mechanical removal of the zona pellucida (see p. 135, 1st col, Results, 1st ¶). They teach isolating inner cell masses from the blastocysts and producing embryonic stem cell lines.

Neither Thomson (2001), Thomson (1998), Rijnders or Lazendorf specifically teach propagating human blastocyst-derived stem cell colonies by repeatedly passaging the cells every 4-5 days wherein each passaging step comprises manually dissecting the inner homogenous structure of the human blastocyst-derived stem cell colonies to form pieces of the same and placing the pieces on inactivated fibroblast feeder cells. However, prior to the time of filing of the instant invention, the '607 patent teaches the production of undifferentiated human ES cells, and particularly teaches methods of propagating ES cells every 5-7 days of about 100 cells using mechanical dissociation. In particular, they teach that Ca/Mg free PBS medium was used to reduce cell attachments, and that when cell dissociation is partial, mechanical dissection using a sharp edge of a pipette is used with cutting and isolation of the clumps. They further teach that in an alternative approach, the combined approach of mechanical cutting of the colonies is performed to cut the colonies into about 100 cells, and the sharp pipette was also used to remove differentiated areas of the colonies. The clumps were then detached and picked up by micro-pipette and transferred to a fresh feeder layer. See col. 18, lines 1-27. Additionally, the '607 patent teaches that the fibroblast feeder cells can be of human or mouse origin (col. 9, lines 60-67). Accordingly, the '607 patent provides guidance for the newly added embodiments. In particular, they teach passaging the cells every 5-7 days, using a sharp pipette (manual dissection). Because the '607 patent teaches cutting non-differentiated cells and removing differentiated cells,

this implicitly refers to the "inner homogenous structure" of the cell colonies (*i.e.*, the portion of the colony which would contain undifferentiated ES cells). Although the '607 patent does not explicitly teach cutting the ICM cells into pieces, one of skill would be readily apprised of variously known techniques, such as cell dissociation taught by the '607 patent for propagating cells, for use in isolating the ICM cells.

Accordingly, it would have been obvious for one of ordinary skill in the art, to combine the teachings of Thomson (2001), Thomson (1998), who teach the production of human ES cell lines from human blastocysts, and with the teachings of Rijnders and Lazendorf, and using the methods taught by the '607 patent to propagate the ES cells using mechanical dissection, with a reasonable expectation of success. One of ordinary skill would have been motivated to use the method of identifying fertilized oocytes/embryos of Grade 1 or 2, as taught by Rinjnders to increase the number of embryos that are capable of producing blastocysts. One of skill ordinary skill in the art would have been also motivated to use the methods, taught by Lazendorf *et al.* to identify blastocysts of Grade A or B, in order identify cells which have clearly defined inner cell masses, which would increase the probability of producing a successful ES cell line. One of ordinary skill in the art would have been motivated to use the methods taught by the '607 patent to propagate hES cells because one of skill in the art would be readily apprised of various methods in which to propagate hES cells, including chemical or mechanical means. See also, '607 patent, col. 4, lines 10-18.

The combined teachings of Thomson (2001), Thomson (1998), Rinjnders, Lazendorf and the '607 patent teach the claimed invention because the teach the general methods of selecting particular human fertilized oocytes or blastocysts for the production of human ES cell lines (claims 1-3), they teach that the blastocyst can be spontaneously hatched (claim 5); they teach methods to propagate the cells by mechanical dissection (claims 1-3); wherein the zona pellucida of the blastocyst

has been partially digested (claim 12); with a digestive agent (claim 13); wherein the feeder cells are embryonic feeder cells (claim 16); wherein the feeder cells in steps ii) and iv) are the same or different, and originate from an animal source (claim 17), particularly mouse (claim 18); wherein the feeder cells are mitotically inactivated (claim 19); the characteristics required by the stem cell line (claim 20); wherein the fibroblast feeder cells are either mouse or human embryonic fibroblast feeder cells (claim 62); wherein the feeder cells are human (claim 64) or mouse fibroblasts (claim 65).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 4, 9, 10, 60 and 61 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijnders *et al.,* Lanzendorf *et al.* in further view of and US Pat. No. 6,875,607 (published April 5, 2005; filed November 9, 1999)as applied to claims 1-3, 5, 6, 12, 13, 16-20, 62, 64, 65 above, and further in view of Marshall *et al.* (**Methods in Molecular Biology**: *Isolation and Maintenance of Primate Embryonic Stem Cells* 158: 11-18, January 2001). This is new ground of rejection.

Applicants' arguments, with regard to the non-obviousness of Thomson (2001)) when taken with Thomson (1998) when taken with Rijnders *et al.* and in further view of Lanzendorf *et al.* (see page 21 of the Response) has been addressed above.

Applicants' arguments regarding Marshall, are with respect to techniques utilized in the establishment of Rhesus macaque monkey ES cell lines (see p. 22 of the Response). Applicants argue that the method of Marshall differ from the claimed invention in that they teach mechanical dissection instead of immunosurgery and/or pipetting to isolating the ICM cells, and the time period (4-5 days) in which the cells are passaged. The Examiner has addressed these issues

above, with respect to the newly added '607 document, which discusses mechanical dissection and teaches the time period required by the claims.

Rejection

Thomson (2001), Thomson (1998), Rijnders, Lazendorf and the '607 patent have been described above. They do not teach the specific densities of the feeder cells required by the claims. However, Marshall discuss the isolation and maintenance of primate embryonic stem cells. They specific teach that the mouse embryonic fibroblasts, which are used as feeder cells, should be plated at 50,000 cells/cm^2 (see page 13, Section 3.1, #3). Thus, Marshall teach cell densities that are less than 60,000 (claims 4 and 9); "about" 45,000 cells/cm^2 (claim 10); less than 55,000 cells/cm^2 (claim 60) and "less than 50,000 cells/cm^2 (claim 61). Furthermore, Marshall teach that the cells should be passaged four to six days after immunosurgery (see p. 13, 3.2, #9), and thus, fulfill the limitation of claim 8.

Accordingly, given the combined teachings of Thomson (2001), Thomson (1998); Rijnders, Lazendorf, the '607 patent, and Marshall, it would have been obvious for one of skill in the art to utilize the methods to produce blastocyst-derived stem cell lines, and passage the cells every 4-5 days, at a density of 50,000 cells/cm^2, as taught by Marshall, with a reasonable expectation of success. One of skill in the art would have been sufficiently motivated to utilize this amount of cells, as Marshall provide a specific protocol to maintain primate ES cells, and they state that because primate ES cells require "regular and meticulous attention to detail in all aspects of the culture process", one of skill in the art would turn to their protocol for direction and specific guidance with regard to the culture of primate ES cells. See page 12, 1st ¶.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijnders *et al.*, Lanzendorf *et al.* and in further view of US Pat. No. 6,875,607 (published April 5, 2005; filed November 9, 1999), as applied to claims 1-3, 5, 6, 12, 13, 16-20, 62, 64, 65 above, and further in view of Conner.

Applicants provide no substantive arguments with respect to this rejection. The Examiner has addressed Applicants' arguments above.

Thomson (2001), Thomson (1998), Rijnders, Lanzendorf and the '607 patent are detailed above. They do not specifically teach that the feeder cells are passaged only 3 times, at most. However, Conner provide guidance to the preparation of mouse embryo fibroblast feeder cells, which are used to maintain human stem cells. See Thomson (2001), col. 8, lines 32-40; Thomson (1998), p. 1147, col. 2, #6. In particular, they suggest freezing the MEFs until use (p. 23.2.4, Freezing and Thawing MEFs). In particular, they teach the following:

> "The advantage of using MEFs is that they provide a more consistent source of feeder cells. Early passage cells with reproducible characteristics must be used because they rapidly lose their ability to divide. Long-term propagation of STO cells can lead to changes that result in characteristics that are less favorable for ES cell growth."
> See p. 23.2.6, Commentary, 2nd ¶, emphasis added.

Furthermore, Conner teach that MEFs are primary cells with limited mitotic potential, and that expanding the cells more may work, but the growth rate will decrease (see p. 23.2.7, 1st col., ¶2).

Accordingly, given the combined teachings, it would have been obvious to one of skill in the art, preparing human ES cells, and using embryonic fibroblasts as feeder cells, to use a early passage cell, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to use early passage embryonic fibroblasts, because Conner clearly teach that later passage cells lose the ability to divide, and can have changes in characteristics that are not

conducive to ES cell growth. Although Conner do not specifically teach that the cells should be passaged less than 3 times, this would be well-within the knowledge of the skilled artisan that the less passages the embryonic fibroblasts are subjected to, the more conducive the feeder cells would be to maintain ES cells. Thus, using feeder cells that have been passaged less than 3 times would be well-within the skills of the ordinary artisan. Additionally, because Conner suggest that the MEFs should be frozen prior to use, this provides additional motivation and suggestion that the embryonic fibroblasts should not be subjected to many passages.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claims 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken Rijnders *et al.*, Lanzendorf *et al.* and in further view of US Pat. No. 6,875,607 (published April 5, 2005; filed November 9, 1999), as applied to claims 1-3, 5, 6, 12, 13, 16-20, 62, 64, 65 above, and in further view of Gardner *et al.* (1998), when taken with Gardner (1999).

Applicants provide no substantive arguments with respect to this rejection. The Examiner has addressed Applicants' arguments above.

Thomson (2001), Thomson (1998), Rijnders, Lanzendorf and the '607 patent are detailed above. They do not specifically teach culturing the blastocysts of step ii) or the inner cell mass of step iv) of claim in the presence of an agent, such as hyaluronic acid, to improve attachment of blastocysts. However, prior to the time of the claimed invention, Gardner teach that culturing of blastocysts in hyaluronic acid (hyaluronate) supports an increase in the implantation of blastocysts in IVF. See Abstract. Gardner teach that culturing human embryos in hyaluronate supports a significantly higher implantation rate (see page 155, 1st ¶); and that

hyaluronate appears to be involved in the attachment of the blastocyst (see p. 155, 2nd ¶). Although Gardner's techniques are used in producing blastocysts that would be used in IVF, the fact that they show an improvement in attachment of the blastocyst is significant. They teach that studies in mice and cattle show that there is a relationship between the rate and normality of nutrient utilization and developmental potential, and that conventional embryo culture causes significant trauma in the developing embryo. Gardner (1999) provides teachings to show that, in mice, blastocyst cell numbers and overall development increased when embryos were cultured in hyaluronan. See Abstract.

Accordingly, in view of the combined teachings, it would have been obvious for one of skill in the art to modify the methods of Thomson (2001), Thomson (1998), Rijnders, Lanzendorf, and the '607 patent, by culturing either the blastocyst of step ii) or the ICM cells of step iv) in a culture medium that contained hyaluronic acid, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to make this modification, as Gardner and Gardner (1999) provide guidance to show that culture medium that contains hyaluronic acid increases attachment of the blastocyst.

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

Claim 35 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson (**U.S. Pat. No., 6,200,806 B1**, issued March 13, 2001, cited above) when taken with Stratagene Catalog, 1988, p. 39.

Applicants provide no substantive arguments with respect to this rejection. The Examiner has addressed Applicants' arguments above.

Accordingly, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine the method of Thomson into a kit format as discussed by Stratagene catalog since the Stratagene

catalog teaches a motivation for combining reagents of use in an assay into a kit, "Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. Thus one need not purchase gram quantities of 10 different reagents, each of which is needed in only microgram amounts, when beginning a series of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually far more expensive for a small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantities you will actually need, premixed and tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control" (page 39, column 1).

Thus, the claimed invention, as a whole, is clearly *prima facie* obvious in the absence of evidence to the contrary.

The prior rejection of claim 64 under 35 U.S.C. 103(a) as being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijnders *et al.* and in further view of Lanzendorf *et al.* in further in view of Xu *et al.* is withdrawn.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thaian N. Ton whose telephone number is (571)272-0736. The examiner can normally be reached on 9-5:30 M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on 571-272-4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Thaian N. Ton/
Primary Examiner, Art Unit 1632